Genetic Mapping of Genes Controlling Partial Resistance and Major Gene Resistance in Sugar Pine

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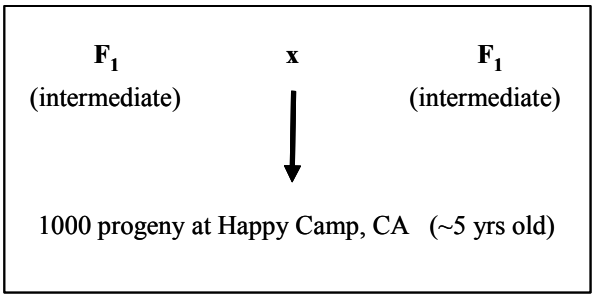
Pines belonging to the *Pinus* subgenus *Strobus* are susceptible to a fungal pathogen (*Cronartium ribicola*) that was introduced to Northern America in the early 1900s. Surprisingly, several white pines have been shown to possess innate resistance to the rust infection. Two forms of resistance have been observed: 1) monogenic (or qualitative) resistance, segregating as a single dominant gene (major gene resistance – MGR) and 2) polygenic (or quantitative) resistance, showing a wide distribution in disease phenotypes (partial resistance). Breeding programs based on deployment of the major gene of resistance are currently in place for sugar pine (*Pinus lambertiana*), however, resistance controlled by a suite of genes may provide resistance that is more durable over time. The number of genetic factors controlling a polygenic trait influences the rate at which genetic gain can be obtained through artificial selection. Hence, knowledge about the number of genes involved in partial resistance is important for any breeding strategy. Genetic mapping of polygenic traits (spring and fall cold-hardiness, dormancy, dormancy-break, wood density, and fiber angle) have been successful in several species from the Pinaceae, providing information about not only the number of genes controlling a trait, but also the location within the genome of such genes, the size of effect that each gene has on a phenotype and whether its mode of action is additive or dominant (Brown et al. 2003; Chagne et al. 2003; Devey et al. 2004; Jermstad et al. 2001a, 2001b, 2003; Neale et al. 2002; Wheeler et al. 2005)

Using sugar pine as a representative species, we are employing several strategies to develop genomic resources for the five-needled pines. The quantitative trait loci (QTL) involved in partial resistance will be mapped in a large full-sib sugar pine family (n>1000) that is segregating for this type of resistance (**Fig.1**). Currently, only the genomic region containing the

Figure 1. Full-sib sugar pine family designed for mapping QTL for partial resistance to *C. ribicola*.



MGR has been mapped in sugar pine (Harkins et al. 1998), thus the construction of a full genome map is much needed. Single nucleotide polymorphisms (SNPs) identified in 1200 gene sequences (see project description at https://www.fastlane.nsf.gov/servlet/showaward?award= 0638502) will provide the marker segregation data required for genetic mapping and will also be a useful resource for nucleotide diversity studies and comparative genomics among conifers.

The genome organization among pines and other related conifers is conserved according to the results of comparative mapping studies (Krutovsky et al. 2004; Neale and Krutovsky 2004). Therefore, it is expected that the genomic architecture and gene sequences of the white pines will be very similar and, therefore, map information and DNA sequences will be transferable among them. In addition to mapping QTL for partial resistance, we are also pursuing a positional cloning strategy to isolate the DNA sequence (allele) conferring MGR. Two RAPD markers flanking the MGR in sugar pine (**Fig. 2**) have been successfully converted to Sequence Characterized Amplified Region (SCAR) markers. Analyses of these SCAR markers in

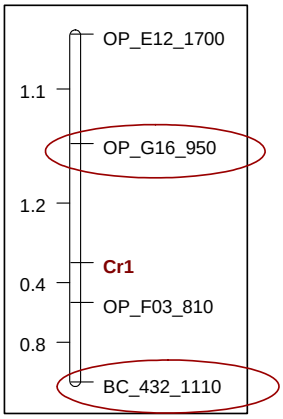
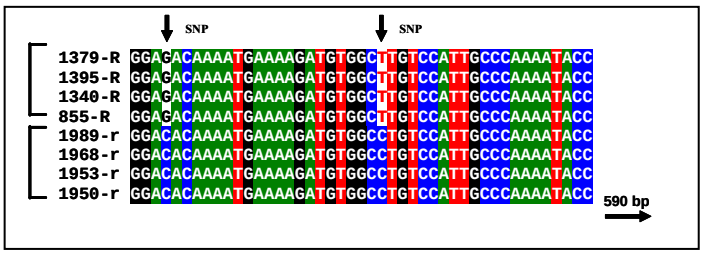


Figure 2. RAPD markers flanking the MGR (*Cr*1) in sugar pine tree 5701 (Harkins et al. 1998). Markers OP_G16_950 and BC_432_1110 are each 1.2 cM (map units) from *Cr*1.

megagametophytes from multiple sugar pine trees revealed SNPs segregating with the disease phenotype (**Fig. 3**), however, each tree examined had a different set of segregating SNPs.

Figure 3. DNA sequences of SCAR marker OPG16$_{950}$ in mega-gametophytes from tree 5701.



R = resistant
r = susceptible

Hence, these markers would have utility for identification of resistant progeny within specific families but would not be applicable for assays in natural populations.

In an effort to isolate the DNA sequence of *Cr*1, SCAR markers will be utilized as probes for screening a loblolly pine BAC library available (http://www.pine.msstate.edu/bac.htm). Isolation of the DNA sequence of the allele conferring MGR in sugar pine (*Cr*1) will provide a precise diagnostic tool for identifying resistant trees in wild populations. Additionally, the isolation of MGR will provide an avenue by which molecular and biochemical interactions between the host and pathogen can be explored.

Because of observed similarity between the genomes of various pines, we expect discoveries pertaining to *C. ribicola* in sugar pine to have direct application in related five-needle pines.

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